AD_____

Award Number: DAMD17-01-1-0132

TITLE: To Ascertain Distinctive Gene Expression Patterns for the Prediction of Docetaxel (Taxotere) Chemosensitivity or Chemoresistance in Human Breast Cancer

PRINCIPAL INVESTIGATOR: Jenny C. Chang, M.D.

CONTRACTING ORGANIZATION: Baylor College of Medicine
Houston, Texas 77030

REPORT DATE: October 2003

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 2003	Annual (17 Sep 2002 - 16 Sep 2003)

4. TITLE AND SUBTITLE
To Ascertain Distinctive Gene Expression Patterns for the Prediction of Docetaxel (Taxotere) Chemosensitivity or Chemoresistance in Human Breast Cancer

5. FUNDING NUMBERS
DAMD17-01-1-0132

6. AUTHOR(S)
Jenny C. Chang, M.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Baylor College of Medicine
Houston, Texas 77030

E-Mail: jcchang@bcm.tmc.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
Original contains color plates: All DTIC reproductions will be in black and white.

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

Chemotherapy is of proven benefit in reducing the risk of death for a subset of patients with early breast cancer, but doctors have problems deciding exactly who should receive this therapy, and which therapy will be most effective for a given patient. As a result, some patients needlessly receive chemotherapy. Even in those patients who clearly require chemotherapy, doctors cannot identify those patients whose tumors might not be responsive to a particular chemotherapy drug. Chemotherapy is also associated with high costs and toxicity including nausea, vomiting, damage to nerves, etc. and increased risk of infections that are sometimes life threatening. The emerging cDNA array technology provides a means to comprehensively appreciate genetic variations in different breast tumors, and may be utilized as a test for chemotherapy sensitivity. Taxotere has one of the highest response rates in breast cancer, and is widely prescribed for the treatment of breast cancer. The aims of this study are therefore, to investigate and validate differential gene expression patterns from core biopsies from patients whose breast tumors either shrank after Taxotere chemotherapy, or failed to respond. These genes whose expression patterns are associated with Taxotere response could be used to create a simple test of predictor genes to help doctors treat breast cancer more effectively.

14. SUBJECT TERMS
Breast cancer

15. NUMBER OF PAGES
17

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

Subject: Annual Report for Award Number DAMD17-01-1-0132 for 09/17/02 – 09/16/03

Introduction

Optimal systemic treatment after breast cancer is the most crucial factor in reducing mortality in women with breast cancer. Adjuvant chemotherapy and hormonal treatment both reduce the risk of death in breast cancer patients. However, while estrogen receptors status predicts for response to hormone treatments, there are no clinically useful predictive markers for chemotherapy responses. All eligible women are therefore treated in the same manner. Even denoval drug resistance will result in treatment failures in many breast cancer patients. Currently, there are no methods available to distinguish those patients who are likely to respond to specific chemotherapies, and given the accepted practice of prescribing adjuvant treatment to most parties, even if the average expected benefit is slow, the selection of appropriate patients represents a major advance in the clinical management of breast cancer today.

We therefore set out to identify gene expression patterns in primary breast cancer specimens that might predict response to taxenes. Neoadjuvant chemotherapy allows for the sampling of the primary tumor for gene expression analysis and for direct assessment of response to chemotherapy by following changes in tumor size during the first few months of treatment. Hence, neoadjuvant chemotherapy provides an idea platform to rapidly discover predictive markers of chemotherapy response.

In this present study, we hypothesize through high quantitation of gene expression, grade is possible to access thousands of genes simultaneously, and expression patterns in different breast cancers might correlate with and thereby predict response to treatment. The purpose of this study was to (1) demonstrate that sufficient RNA could be obtained from core biopsies to access gene expression, (2) to identify groups of genes that could be used to distinguish primary breast cancers to responsive or resistance to taxotere, and (3) to identify gene pathways that could be important in a mechanism of action of taxotere.

Body of Research

From September 17, 2001 to September 16, 2003, we had recruited 65 patients with locally advanced breast cancer. Core biopsies were obtained from the primary breast cancers before commencement of neoadjuvant chemotherapy. Clinical responses before and after four cycles of chemotherapy were measured in all primary breast cancers.

A total of 6 core biopsies were obtained from each primary cancer. Two core biopsy specimens were transferred immediately to liquid nitrogen and snap frozen at -80° C. Each core biopsy measured approximately 1 cm x 1 mm. As these core biopsies were too small for micro dissection, we ascertained the tumor cellularity of the pretreatment core biopsies. In general, the core biopsies showed good tumor cellularity with median tumor cellularity of 75% (range 40-

100%). Each core biopsy yielded 3-6 mg of total RNA, which is more than sufficient to generate approximately 20 mg of label cRNA needed for hybridization with the Affymetrix U95Av2 Genechip, using the manufacturer's standard protocols. To date, we have analyzed 24 out of the 42 collected core biopsy specimens. We compared the expression data in sensitive and resistant tumors to identify gene significance differentially expressed between the two groups. We applied filtering to eliminate genes with uniformly low expression or whose expression did not vary significantly across the samples retaining approximately 1,600 genes. We then applied T-test after lot transformation, to select discriminatory genes. To date, we have selected 92, 300, 551 genes as differentially expresses at p values of 0.001, 0.01, 0.05, respectively.

In the 92 gene list, among the genes overexpressed in the resistant cluster, most are involved in transcriptional regulation, signal transduction, or have unknown functions. In the sensitive tumors, some are involved in signal transduction and cell cycle, cytoskeleton and adhesion processes, protein transport, protein modification, stress and apoptosis or have unknown functions. We have confirmed the expression measurements obtained from the affymetrics chips with values from semiquantitative RT-PCR. We have done 15 genes and compared their measurements with QRT PCR. Significantly correlation was seen between the two methods.

Key Research Accomplishments

Four abstracts have been submitted and accepted for international meetings. Two were submitted to the San Antonio Breast Cancer Symposium in 2001 and 2002. This abstract was also submitted to the ASCO meeting in 2002 and 2003. This study was also selected for a preliminary presentation in the Era of Hope Meeting in Florida in 2002. A manuscript has been recently published in the prestigious medical jurnal, *The Lancet.*

Reportable Outcomes

1. Genetic markers for response to neoadjuvant therapy: Array based gene expression profiling from serial biopsies. EC Wooten, **J Chang**, SG Hilsenbeck. 24th Annual San Antonio Breast Cancer Symposium, San Antonio, Texas (abstract 236), December 2001.

2. Gene expression profiles from breast cancer core biopsies predict therapy to response. EC Wooten, **J** Chang, SG Hilsenbeck. *Proceedings of the American Association for Cancer Research* 43, abstract 450, March 2002.

3. Gene expression profiles for doxytaxcil chemosensitivity. **J Chang,** EC Wooten and R Elledge. ASCO 28th Annual Meeting, abstract 1700, May 2002.

4. **JC Chang**, EC Wooten, A Tsimelzon, SG Hilsenbeck, MC Gutierrez, R Elledge, S Mohsin, CK Osborne, GC Chamness, DC Allred, P O'Connell. Gene expression profiling for the prediction of therapeutic response to docetaxel in patients with breast cancer. *The Lancet* 362:362-369, 2003.

Conclusions

We have determined that 1) sufficient RNA can be obtained from core needle biopsies to hybridize Affymetrix GeneChips for assessment of gene expression patterns 2) differential gene expression patterns exist that can distinguish resistant *versus* sensitive tumors. We will continue our current experiments to further increase patient recruitment to define and refine patterns of resistance and sensitivity.

References

1. EBCTG. Systemic treatment of early breast cancer by hormonal, cytotoxic or immune therapy: 133 randomised trials involving 31,000 recurrences and 24,000 deaths among 75,000 women. *Lancet* **339**, 1-15, 71-85 (1992).
2. The Early Breast Cancer Trialists' Collaborative Group, E. Tamoxifen for early breast cancer: an overview of the randomised trials. *Lancet* **351**, 1451-1467 (1998).
3. EBCTG. Polychemotherapy for early breast cancer: an overview of the randomised trials. *Lancet* **352**, 930-942 (1998).
4. Hortobagyi, G.N. Docetaxel in breast cancer and a rationale for combination therapy. *Oncology* **11**, 11-15 (1997).
5. Chan, S. et al. Prospective randomized trial of docetaxel versus doxorubicin in patients with metastatic breast cancer. The 303 Study Group. *J Clin Oncol* **17**, 2341-54. (1999).
6. Nabholtz, J.M. et al. A phase III trial comparing docetaxel (T), doxorubicin (A) and cyclophosphamide (C) (TAC) to (FAC) as first line chemotherapy for patients with metastatic breast cancer. *Proceedings of the American Society of Clinical Oncologists* **20**, 22a (2001).
7. Henderson IC, B.D., Demetri G, et al. Improved disease free survival and overall survival from the addition of sequential paclitaxel but not from escalation of doxorubicin in the adjuvant chemotherapy of patients with node-positive primary breast cancer. *Proc Am Soc Clin Onco* **17**, 101 (1998).
8. Aapro, M.S. Adjuvant therapy of primary breast cancer: a review of key finding s from the 8th international conference, St. Gallen. *The Oncologist* **6**, 376-385 (2001).
9. Fisher, B. et al. Effect of preoperative chemotherapy on the outcome of women with operable breast cancer. *Journal of Clinical Oncology* **16**, 2672-2685 (1998).
10. Mamounas, E.P. Preoperative doxorubicin plus cyclophosphamide followed by preoperative or postoperative docetaxel. *Oncology* **11**, 37-40 (1997).
11. Schena, M., Shalon, D., Davis, R.W. & Brown, P.O. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* **270**, 467-470 (1995).
12. Khan, J. et al. Gene expression profiling of alveolar rhabdomyosarcoma with cDNA microarrays. *Cancer Research* **58**, 5009-5013 (1998).

13. Lockhart, D.J., Dong, H., Byrne, M.C. & et al. Expression monitoring by hybridization to high-density oligonucleotide arrays. *Nature Biotechnology* **14**, 1675-1680 (1996).
14. Sgroi, D.C. et al. In vivo gene expression profile analysis of human breast cancer progression. *Cancer Research* **59**, 5656-5661 (1999).
15. Perou, C.M. et al. Distinctive gene expression patterns in human mammary epithelial cells and breast cancers. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 9212-9217 (1999).
16. Li, C. & Wong, W.H. Model-based analysis of oligonucleotide arrays: expression index computation and outlier detection. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 31-36 (2001).
17. Schadt, E.E., Li, C. & Wong, W.H. Analyzing high-density oligonucleotide gene expression array data. *Journal of Cellular Biochemistry* **80**, 192-202 (2001).
18. Tusher, V.G., Tibshirani, R. & Chu, G. Significance analysis of microarrays applied to the ionizing radiation response. *Proc Natl Acad Sci U S A* **98**, 5116-21. (2001).
19. Hakak, Y. et al. Genome-wide expression analysis reveals dysregulation of myelination-related genes in chronic schizophrenia. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 4746-4751 (2001).
20. Bowcock, A.M. et al. Insights into psoriasis and other inflammatory diseases from large-scale gene expression studies. *Human Molecular Genetics* **10**, 1793-1805 (2001).
21. Van Poznak, et al. Assessment of molecular markers of clinical sensitivity to single agent taxane therapy for metastatic breast cancer. *Journal of Clinical Oncology* **20(9)**, 2319-2326 (2002)
22. Hansen, R.K. et al. Hsp27 overexpression inhibits doxorubicin-induced apoptosis in human breast cancer cells. *Breast Cancer Res Treat* **56**, 187-96. (1999).

THE LANCET
32 Jamestown Road London NW1 7BY UK

Gene expression profiling for the prediction of therapeutic response to docetaxel in patients with breast cancer

Jenny C Chang
Eric C Wooten
Anna Tsimelzon
Susan G Hilsenbeck
M Carolina Gutierrez
Richard Elledge
Syed Mohsin
C Kent Osborne
Gary C Chamness
D Craig Allred
Peter O'Connell

Reprinted from THE LANCET
2 August 2003 Vol. 362
No. 9381 Pages 362-369

Mechanisms of disease

Gene expression profiling for the prediction of therapeutic response to docetaxel in patients with breast cancer

Jenny C Chang, Eric C Wooten, Anna Tsimelzon, Susan G Hilsenbeck, M Carolina Gutierrez, Richard Elledge, Syed Mohsin, C Kent Osborne, Gary C Chamness, D Craig Allred, and Peter O'Connell

Summary

Background Systemic chemotherapy for operable breast cancer substantially decreases the risk of death. Patients often have de novo resistance or incomplete response to docetaxel, one of the most active agents in this disease. We postulated that gene expression profiles of the primary breast cancer can predict the response to docetaxel.

Methods We took core biopsy samples from primary breast tumours in 24 patients before treatment and then assessed tumour response to neoadjuvant docetaxel (four cycles, 100 mg/m^2 daily for 3 weeks) by cDNA analysis of RNA extracted from biopsy samples using HgU95-Av2 GeneChip.

Findings From the core biopsy samples, we extracted sufficient total RNA (3–6 μg) for cDNA array analysis using HgU95-Av2 GeneChip. Differential patterns of expression of 92 genes correlated with docetaxel response (p=0·001). Sensitive tumours had higher expression of genes involved in cell cycle, cytoskeleton, adhesion, protein transport, protein modification, transcription, and stress or apoptosis; whereas resistant tumours showed increased expression of some transcriptional and signal transduction genes. In leave-one-out cross-validation analysis, ten of 11 sensitive tumours (90% specificity) and 11 of 13 resistant tumours (85% sensitivity) were correctly classified, with an accuracy of 88%. This 92-gene predictor had positive and negative predictive values of 92% and 83%, respectively. Correlation between RNA expression measured by the arrays and semiquantitative RT-PCR was also ascertained, and our results were validated in an independent set of six patients.

Interpretation If validated, these molecular profiles could allow development of a clinical test for docetaxel sensitivity, thus reducing unnecessary treatment for women with breast cancer.

Lancet 2003; **362:** 362–69
See Commentary page 340

Breast Center and the Departments of Medicine, Pathology, and Molecular and Cellular Biology, Baylor College of Medicine, and the Methodist Hospital, Houston, TX USA (J C Chang MD, EC Wooten PhD, A Tsimelzon PhD, Prof S G Hilsenbeck PhD, M C Gutierez MD, R Elledge MD, S Mohsin MD, Prof C K Osborne MD, Prof G C Chamness PhD, Prof D C Allred MD, Prof P O'Connell PhD)

Correspondence to: Dr Jenny Chang, Breast Center, Baylor College of Medicine, One Baylor Plaza, BCM 600, Houston, TX 77030, USA (e-mail: jcchang@breastcenter.tmc.edu)

Introduction

Adjuvant systemic treatment after surgery for breast cancer is the most crucial factor in reducing mortality—both chemotherapy and hormonal treatment reduce the risk of death in such patients.[1–4] However, although oestrogen-receptor status is predictive of response to hormonal treatments, there are no clinically useful predictive markers of a patient's response to chemotherapy. Therefore, all patients who are eligible for chemotherapy receive the same treatment, even though de novo drug resistance will result in treatment failures in many. The taxanes, docetaxel and paclitaxel, are a new class of antimicrotubule agent that are more effective than older drugs such as anthracyclines,[5–7] although results of clinical trials with taxanes and anthracyclines in combination show that only a small subset of patients benefit from the addition of taxanes.[8,9] There are no methods to distinguish between patients who are likely to respond to taxanes and those who are not. In view of the accepted practice of giving adjuvant treatment to most patients, even if the average expected benefit is low, the a priori selection of appropriate patients most likely to benefit from adjuvant treatment with taxanes would be a great advance in the clinical management of breast cancer.[8,9] A major impediment in the study of predictors of effectiveness of adjuvant treatment is the absence of surrogate markers for survival and, consequently, large numbers of patients and long-term follow-up are needed.

We aimed to identify gene expression patterns in primary breast-cancer specimens that might predict response to taxanes. Neoadjuvant chemotherapy (ie, treatment before primary surgery) allows for sampling of the primary tumour for gene expression analysis, and for direct assessment of response to chemotherapy by monitoring changes in tumour size during the first few months of treatment.[10,11] Clinical response of the tumour to neoadjuvant chemotherapy is a valid surrogate marker of survival: patients whose tumours regress substantially after neoadjuvant chemotherapy have better outcome than do those with modest response or clinically obvious disease that is resistant to chemotherapy.[10,11] With the advent of high-throughput quantification of gene expression, simultaneous assessment of thousands of genes is now possible, which allows identification of expression patterns in different breast cancers that might correlate with, and thereby predict, excellent clinical response to treatment.[12–16] These profiles have potential to explain the genetic heterogeneity of breast cancer and allow treatment strategies to be planned in accordance with their probability of success in individual patients. Hence, neoadjuvant chemotherapy provides an ideal platform from which to discover predictive markers of chemotherapy response. In our study, we took core needle biopsy samples of the primary breast cancer for gene expression profiling before patients received neoadjuvant docetaxel. We aimed first, to show that sufficient RNA

GLOSSARY

ANEUPLOIDY
Cells containing an abnormal complement of chromosomes.

APOPTOSIS
Programmed cell death. A genetic mechanism leading to induced cell death that involves activation of a cascade of genes. Apoptosis arises in normal tissue and can be associated with particular disease states.

RESUBSTITUTION ESTIMATES
Application of the classifier to the samples used to create it.

could be obtained from core biopsy samples to assess gene expression; second, to identify groups of genes that could be used to distinguish primary breast cancers that are responsive or resistant to docetaxel chemotherapy; and third, to identify gene pathways that could be important in the mechanism of resistance to docetaxel.

Methods

Patients

From September, 1999, to June, 2001, patients with locally advanced breast cancer (ie, primary cancers >4 cm, or clinically evident axillary metastases) were considered for a phase II study with neoadjuvant docetaxel. Inclusion criteria were (1) age greater than 18 years and a diagnosis of breast cancer confirmed by analysis of a core needle biopsy sample, (2) premenopausal status accompanied by appropriate contraception, (3) adequate performance status, and (4) adequate liver and kidney function tests (all within 1·5 times the institution's upper limit of normal). Patients were excluded if they had severe underlying chronic illness or disease, or were taking other chemotherapeutic drugs while on study.

This study (protocol H8448) was approved by the institutional review board of Baylor College of Medicine, Houston, TX, USA. Patients gave written informed consent.

Clinical procedures

We recorded clinical staging and size of primary tumour at the start of treatment, at every cycle, and after completion of four cycles of chemotherapy. Tumour size (product of the two largest perpendicular diameters) measured before and after four cycles of neoadjuvant chemotherapy was used to calculate the percentage of residual disease. The median residual disease was then calculated, and this degree of response was used to divide the cancers into two roughly equal groups—sensitive and resistant tumours—before we did gene expression analysis.

Before docetaxel was given, we did core biopsies of the primary cancers. To obtain sufficient tissue, we did about six core biopsies from every patient using an MC1410 MaxCore biopsy instrument (Bard, Covington, GA, USA). Samples were taken after patients had been given local anesthesia with the same entry point, but reorienting the needle. Two to three core biopsy specimens were immediately transferred for snap freezing at −80°C for cDNA array analysis. The remaining specimens were fixed in formalin for diagnostic analysis and possible immunohistochemical analysis.

Four cycles of docetaxel were given at 100 mg/m^2 every 3 weeks, and we assessed clinical response after the fourth cycle, at 12 weeks. As part of standard care, patients were continued on neoadjuvant chemotherapy through the full four cycles, unless there was clear documentation of progressive disease, which we defined as an increase in tumour size of more than 25%. After the course of neoadjuvant docetaxel was complete, primary surgery was done and standard adjuvant treatment was given.

RNA extraction and amplification

We isolated total RNA from the frozen core biopsy specimens in accordance with protocols recommended by Affymetrix (Santa Clara, CA, USA) for GeneChip experiments. Total RNA was isolated with Trizol reagent (Invitrogen Corporation, Carlsbad, CA). Samples were subsequently passed over a Qiagen RNeasy column (Qiagen, Valencia, CA) for removal of small fragments that affect RT-reaction and hybridisation quality (ECW, unpublished data). Each core biopsy yielded 3–6 μg of total RNA. After RNA recovery, double-stranded cDNA was then synthesised by a chimeric oligonucleotide with an oligo-dT and a T7 RNA polymerase promoter at a concentration of 100 pmol/μL.

We did reverse transcription in accordance with protocols recommended by Affymetrix using commercially available buffers and proteins (Invitrogen Corporation). Biotin labelling and about 250-fold linear amplification followed phenol-chloroform clean up of the reverse-transcription reaction product and was done by in-vitro transcription (Enzo Biochem, New York, NY, USA) over a reaction time of 8 h. From each biopsy specimen, we hybridised 15 μg of labelled cRNA onto the HgU95-Av2 GeneChip using recommended procedures for pre-hybridisation, hybridisation, washing, and staining with streptavidin-phycoerythrin (SA-PE). Antibody amplification was done with a biotin-linked antibody to streptavidin (Vector Laboratories, Burlingame, CA) with a goat-IgG blocking antibody (Sigma, St Louis, MO, USA). A second application of the SA-PE dye was used after additional wash steps had been done. After automated staining and wash protocols (Affymetrix protocol EukGE-2v4), the arrays were scanned by the Affymetrix GeneChip scanner (Agilent, Palo Alto, CA) and quantitated with Micoarray suite version 5.0 (Affymetrix). The HgU95-Av2 GeneChip consists of about 12 625 probe sets, each containing about 16 perfect match and corresponding mismatch 25mer oligonucleotide probes representing sequences (genes), most of which have been characterised in terms of function or disease association. The raw, un-normalised probe level data were then analysed by dChip (http://dchip.org) for final normalisation and modelling. Median intensity was used for the normalisation of the 24 arrays and the perfect match/mismatch (PM/MM) modelling algorithm was used.

Semiquantitative RT-PCR

We did semi-quantitative RT-PCR (sqRT-PCR) measurement of gene expression levels using the same amplified cRNA hybridised to the GeneChip. 20 genes were selected for analysis on the basis of their high variation in expression. Primers were designed for these loci with the sequences freely available from the Entrez Nucleotide database[17] and the Primer3 algorithm for primer design. Product sizes were kept short (<150 bp) to allow the maximum ability to work under varying conditions relative to cRNA quality. Primers were optimised with a reverse-transcribed mixture of six samples. 15 duplicate reactions were prepared and samples were taken at alternating cycle numbers between 15 and 33 to ensure that the sqRT-PCR reaction products were in a linear range of accumulation. These samples were then arranged in ascending order, diluted with 10 μL loading buffer, and 3 μL of each sample was loaded onto 6% denaturing acrylamide gels. Electrophoresis at 60 W was done for 2 h, or until sufficient separation of the xylene cyanol and bromophenol blue dyes

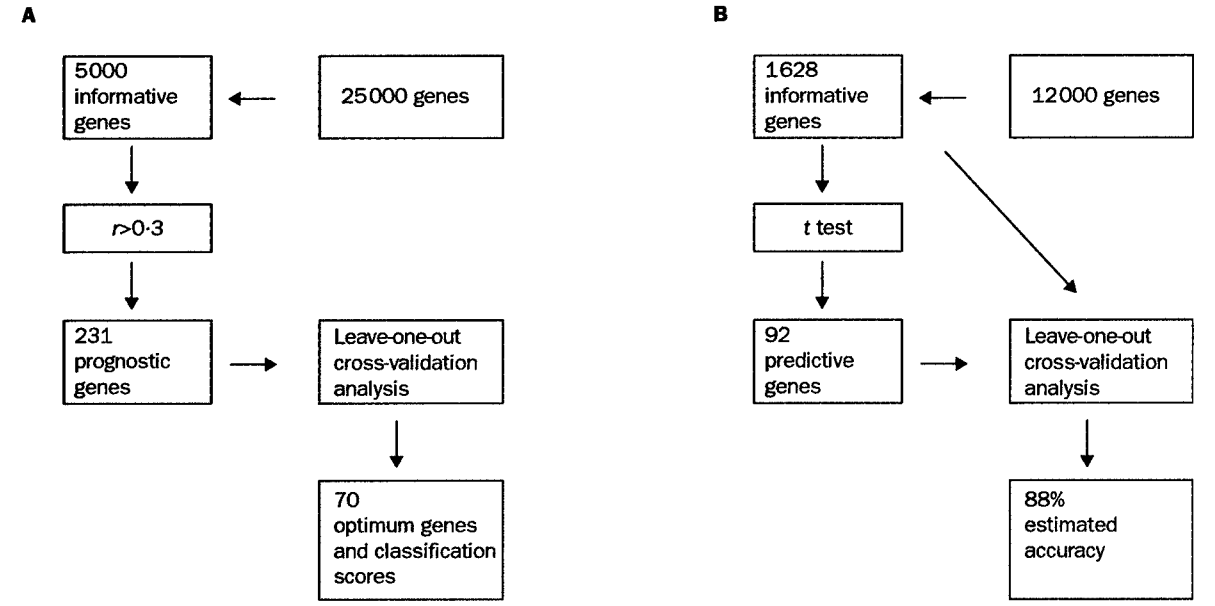

Figure 1: **Two methods of statistical analysis**

A: the prognostic analysis used by van't Veer and colleagues[18] used oligonucleotide microarrays with 25 000 genes, from which 5000 variably expressed genes were selected by filtering. Of these, 231 genes were significantly associated with prognostic outcome ($r>0·3$). These 231 genes were then rank-ordered on the basis of the magnitude of the correlation coefficient and selected in groups of five to construct the smallest optimum classifier. Leave-one-out analysis was then done with 231 genes that were correlated with outcome to select a classification set of 70 genes. B: statistical analysis methods used in this study: a subset of 1628 genes was selected by filtering on signal intensity to eliminate genes with uniformly low expression or genes whose expression did not vary significantly across the samples.

was achieved. Gels were then fixed, removed from the rear plate, transferred to filter paper, and dried. We first assessed these dry gels using autoradiography (about 8 h exposure, no intensification), and analysable gels were then exposed to phosphorimaging screens. Primers that failed to produce a single clear band were used again with different annealing temperatures until a single band was produced.

15 of the 20 primers chosen proved suitable to use and gave clean, single bands for analysis. The remaining five failed to optimise properly and were not included in any further analysis. Although high-cycle samples inevitably achieved pixel-saturation, care was taken to keep exposure times to a minimum, so as to keep intensity within the informative range on most cycle-totals within each set. To determine the linear range of the 15 primers, we analysed their absolute intensities using Microsoft Excel graphing functions. We then did phosphorimager quantification analysis (Bio-Rad Laboratories, Hercules, CA), and RT-PCR product band intensities were quantitatively compared with normalised, model-based estimates of expression from the GeneChip data.

Statistical analysis

The analytical approach used in this study (figure 1) was similar to the successful methods described previously.[18] After scanning and low-level quantification using Microarray Suite (Affymetrix), we used DNA-Chip analyser dChip version 1.2 to adjust arrays to a common baseline[19] and estimated expression using Li and colleagues' PM-MM model.[20,21] We eliminated genes that were not present in at least 30% of samples, and exported expression data for the remaining 6849 genes to BRB Arraytools version 2.1c[22] for more filtering and analysis. After transforming all data by taking logarithms, we ranked genes by variability over all 24 samples, and we retained the 1628 genes that were significantly more variable than the median variance.

We selected differentially expressed genes from the filtered gene list using the two-sample t test, and then used a global permutation test as an overall, multiple comparison-free test of whether the number of differentially expressed genes exceeded that which might arise by chance. In this test, the observed number of significantly differentially expressed genes was compared with the distribution of numbers of differentially expressed genes generated by repeatedly permutating the labels of the samples and recalculating the t test at the specified level of significance.

Next, we developed a classifier to predict response. With a list of discriminatory genes and their associated t values, we used the compound covariate predictor method of Radmacher and colleagues.[23] to construct a linear classifier. RESUBSTITUTION ESTIMATES of classification success, in which the classifier is applied to the same samples used to create it, are invariably biased (ie, they are overly optimistic).[24,25] Therefore, we used an external cross-validation procedure to generate a less biased estimate of classification success. Starting with 1628 genes that had significant variation in expression, and which were filtered without any respect to class membership, the entire gene selection and classifier construction process was repeated in a leave-one-out cross-validation to estimate classifier performance. Finally, to assess whether the degree of successful classification we noted could have arisen by chance, the entire cross-validation procedure was repeated 2000 times, permutating the sample labels every time. The observed cross-validated classification success rate was then compared with the distribution of classification success in the permutation analysis. Cross-validated performance was summarised by observed sensitivity and specificity, and associated exact binomial confidence intervals. Resubstitution classifier values were also used to generate a receiver operating characteristic curve (ROC curve) and to estimate the area under the curve.

The classifier was partly validated with an independent set of six patients treated in the same clinical trial as those

Patient	Age (years)	Menopausal status	Ethnic origin	Bidimensional tumour size (cm)	Clinical axillary nodes	Oestrogen-receptor status	Progesterone-receptor status	HER-2	Tumour type
1	37	Premenopausal	Hispanic	10×10	No	–	–	–	IMC
2	55	Postmenopausal	Hispanic	10×8	Yes	–	–	+	IDC
3	41	Premenopausal	Black	6×5	Yes	+	+	–	IDC
4	43	Premenopausal	Black	15×13	Yes	+	–	–	IMC
5	50	Postmenopausal	Black	20×23	Yes	–	–	–	IDC
6	55	Postmenopausal	Black	11×11	Yes	+	+	–	IDC
7	42	Premenopausal	Black	7×9	Yes	+	+	–	IMC
8	63	Postmenopausal	Black	7×8	Yes	+	+	–	IMC
9	50	Postmenopausal	Black	13×9	No	+	+	–	IDC
10	38	Premenopausal	Hispanic	8×8	Yes	+	+	–	IMC
11	58	Postmenopausal	Hispanic	7×7	Yes	+	+	–	IMC
12	62	Postmenopausal	Hispanic	4×4	Yes	+	–	–	IDC
13	40	Premenopausal	Hispanic	5·5×4·5	No	+	+	–	IMC
14	36	Premenopausal	Black	6×6	Yes	+	+	–	IDC
15	56	Postmenopausal	Black	5×5·5	No	+	–	–	IMC
16	38	Premenopausal	White	6×6	Yes	+	–	–	IDC
17	54	Postmenopausal	White	5×6	Yes	+	+	+	IDC
18	52	Postmenopausal	White	10×10	No	+	+	–	IDC
19	57	Postmenopausal	White	8×8	No	–	–	–	IDC
20	52	Postmenopausal	Black	10×10	No	–	–	–	IDC
21	44	Premenopausal	Black	11×11	No	–	–	–	IDC
22	41	Premenopausal	Black	6×5	Yes	+	+	–	IDC
23	38	Premenopausal	White	8×8	Yes	+	+	–	IDC
24	54	Postmenopausal	Black	9×7	No	+	+	–	IDC

HER-2=HER-2/neu oncogene detected by immunohistochemical analysis. –=negative. +=positive. IMC=invasive mammary carcinoma. IDC=invasive ductal carcinoma.

Table 1: **Characteristics of patients in the training set**

in the training set. RNA was obtained from pretreatment biopsy samples and hybridised to HgU95-Av2 GeneChips exactly as described for the training sample. Probe level data were adjusted to the same baseline array as the training set, and gene expression values were calculated with previously estimated probe sensitivity values derived from the training sample. The 92-gene classifier was then applied to predict response in every new sample.

Role of the funding source

The study sponsors did not contribute to the study design, or collection, analysis, or interpretation of data. The manuscript was reviewed with only minor editorial changes by one of the study's sponsors, Aventis Pharmaceutical.

Results

Assessment of clinical response

We included 24 patients, and their clinical characteristics are shown in table 1. Unidimensional median tumour size before treatment was 8 cm (range 4–23 cm). Before doing gene expression analysis, we defined tumour sensitivity and resistance on the basis of the percentage of residual disease after treatment. We first determined that the median residual disease after chemotherapy was 30%. We then arbitrarily defined sensitive tumours as those that had 25% or less residual disease, and resistant tumours as those with more than 25% residual disease, since this cutoff divides the patients into two almost equally sized groups for statistical comparison. In this study of locally advanced breast cancer, tumours were large and a regression of at least 75% after chemotherapy would almost certainly represent a clinically important response. Of these 24 patients, 11 (46%) were sensitive to docetaxel and 13 (54%) were resistant. Of the sensitive tumours, five patients (45%) had minimal residual disease (<10% residual tumour), whereas of the resistant tumours, seven (58%) had residual tumour mass of 60% or greater, and three (23%) of these residual tumours were 100% or greater of baseline.

Selection of discriminatory genes

To select discriminatory genes, we compared expression data in the sensitive and the resistant tumours (figure 2). First, we selected a subset of candidate genes by filtering on signal intensity to eliminate genes with uniformly low expression or genes whose expression did not vary significantly across the samples, retaining 1628 genes. After log transformation, a *t* test was used to select discriminatory genes. *t* tests with nominal p values of 0·001, 0·01, and 0·05 selected 92, 300, and 551 genes, respectively, for which expression differed in sensitive and resistant groups—ie, differentially expressed. The probability that these numbers of genes would be selected by chance alone was estimated to be 0·0015, 0·001, and less than 0·001 respectively (table 2). These results can be reviewed with data at the gene expression omnibus.[26]

Functional classification of discriminatory genes

The 92 genes classed as most significantly "differentially expressed" at p=0·001 are listed in the webtable (http://image.thelancet.com/extras/01art11086webtable.pdf) (figure 2). These genes showed 2·6–4·2-fold decreases or 2·5–15·7-fold increases in expression in resistant compared with sensitive tumours. Functional classes of these differentially expressed genes included stress or APOPTOSIS (21%), cell adhesion or cytoskeleton (16%), protein transport (13%), signal transduction (12%), RNA transcription (10%), RNA splicing or transport (9%), cell cycle (7%), and protein translation (3%); the remainder (9%) have unknown functions. 14 of these 92 genes were overexpressed in the treatment-resistant cluster with major categories including unknown function, protein

	p value for gene selection		
	0·001	0·01	0·05
Number of differentially expressed genes	92	300	551
Permutation p*	0·0015	0·001	0·001

*The proportion of permutations in which the number of genes selected exceeds the observed number of genes.

Table 2: **Group comparison analysis, with different nominal p-values**

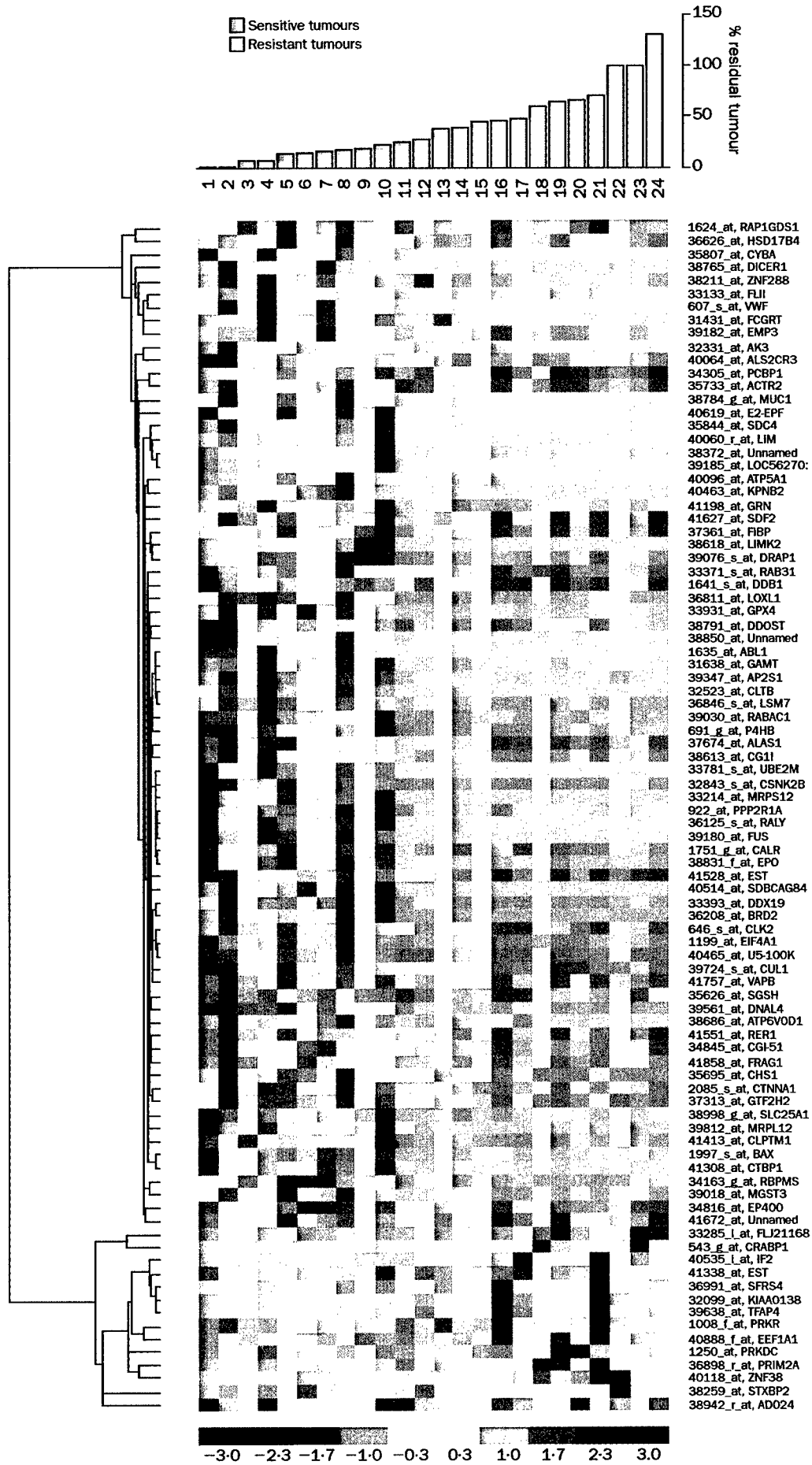

Figure 2: **Hierarchical clustering of genes correlated with docetaxel response**
Expression levels above the mean for the gene are shown in red and expression levels below the mean for the gene are shown in blue. The colour scale ranges from 3 SDs or more below the mean (darkest blue) to 3 SDs above the mean (darkest red). Affymetrix probe set identifiers and gene symbols are shown on the right-hand side of the figure.

translation, cell cycle, and RNA transcription. Tubulin isoforms were associated with docetaxel resistance.

Of the 78 genes overexpressed in docetaxel-sensitive tumours, major categories were stress or apoptosis, adhesion or cytoskeleton (no genes with this function were overexpressed in resistant tumours), protein transport, signal transduction, and RNA splicing or transport. In sensitive tumours, genes involved in apoptosis (eg, overexpression of *BAX*, *UBE2M*, *UBCH10*, *CUL1*), and DNA damage-related gene expression (eg, overexpression of CSNK2B, DDB1, and *ABL1*, and underexpression of *PRKDC*) seem to contribute to docetaxel sensitivity.

Leave-one-out cross-validation

In this cross-validation analysis, we began with all 1628 filtered genes to avoid selection bias.[24,25] Every observation in turn was left out and the remaining samples were used to select differentially expressed genes; we then constructed a compound covariate predictor to classify the left-out sample. Ten of 11 sensitive tumours (91% specificity, [95%CI 0·59–1·00]) and 11 of 13 resistant tumours (85% sensitivity [0·55–0·98]) were correctly classified, for an overall accuracy of 88% (68–97%). Results of permutation testing showed that such a high cross-validated classification accuracy is significant (p=0·008). The analogous predictor, constructed with 92 genes selected with use of all 24 samples, yielded identical classification success. With this predictor, positive and negative predictive values for response to docetaxel were 92% and 83%, respectively, and the area under the ordinary receiver operating characteristic (ROC) curve was 0·96 (figure 3).

Confirmation of expression measurements

To confirm measurement of RNA concentrations, expression values derived from adjusted Affymetrix data were correlated with values from sqRT-PCR for 15 variably expressed genes (table 3). Spearman rank correlations were positive for 13 genes and significantly positive for six of 15 genes.

Validation in an independent cohort

The six additional patients enrolled in this prospective clinical study were studied to partly validate the 92-gene predictive classifier. In this small set, all six patients had sensitive tumours and were correctly classified by our predictive method.

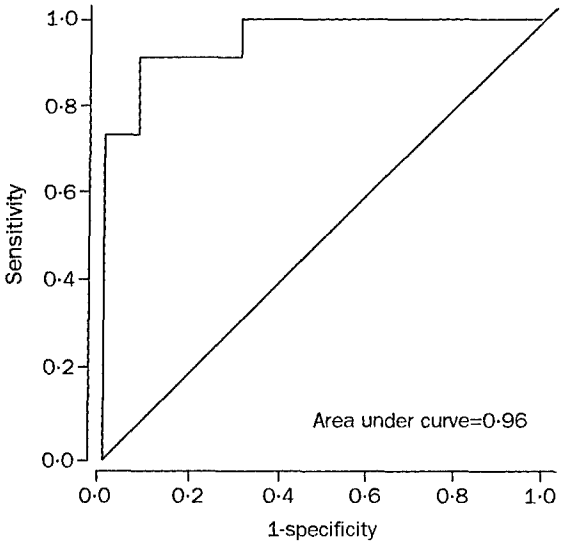

Figure 3: **Receiver operating characteristic (ROC) curve for predicting response to docetaxel**

	Affymetrix probe set	Number	Pearson correlation		Spearman rank correlation	
			r	p	r_s	p
ACTB	32318_s_at	5	0·81	0·09	0·90	0·04
ATP6V0E	33875_at	5	0·28	0·65	0·10	0·87
BMI-1	1728_at	8	0·90	0·002	0·21	0·61
CALM3	1158_s_at	7	0·52	0·23	0·64	0·12
FUCA1	41814_at	6	0·77	0·07	0·94	0·00
GLRX	34311_at	8	0·74	0·03	0·50	0·21
IFITM1	676_g_at	5	0·74	0·15	0·70	0·19
LAMR1	256_s_at	8	0·69	0·06	0·85	0·01
LMNA	37378_r_at	5	−0·08	0·90	−0·40	0·50
MUC1	38783_at	8	0·84	0·01	0·71	0·05
MYO10	35362_at	8	0·15	0·72	0·05	0·91
PLOD	36184_at	4	−0·41	0·59	−0·80	0·20
PSMD5	32240_at	8	0·27	0·52	0·33	0·42
SERPINB5	863_g_at	8	0·75	0·03	0·81	0·01
SPARCL1	36627_at	6	0·92	0·01	1·00	0·00

Correlations positive for 13 genes and significantly positive for 6 of 15 genes

Table 3: **Correlation of Affymetrix expression data with sqRT-PCR derived values.**

Discussion

We obtained sufficient RNA from small core biopsy samples of human breast cancers, to assess patterns of gene expression in individual tumours and identified molecular profiles using gene expression patterns of human primary breast cancers to accurately predict sensitivity to docetaxel in women with primary breast cancer.

Gene expression patterns associated with docetaxel sensitivity and resistance are highly complex. In the past, investigators using single gene biomarkers to assess sensitivity and resistance to chemotherapy have seldom produced conclusive results. For example, in a breast cancer study the researchers did not note any correlation between commonly measured predictive and prognostic markers (HER-2, p53, p27, or epidermal growth factor receptor) and taxane sensitivity.[27] Reports of different cancer types have suggested that alterations in expression levels of β tubulin isoforms might represent an important and complex mechanism of taxane resistance.[28] We noted that overexpression of some β tubulin isoforms was associated with docetaxel resistance in some tumours, but not all. These results suggest that the patterns of gene expression for sensitivity and resistance are likely to involve multiple gene pathways, and that integration of many genes in these pathways leads to drug sensitivity and resistance. Our results lend support to the idea that assessment of expression of a few individual genes will not be powerful enough to untangle the heterogeneity of clinical breast cancers, but that patterns of expression of many genes could be successful in distinguishing between sensitive and resistant tumours.

A key point of this study was to focus on genes that could be reliably measured and to exclude those that were unlikely to be expressed in any sample. We did not design this study to discover specific genes for docetaxel response or resistance, but rather to identify patterns of many genes that could be used as a predictive test in patients with breast cancer. As a result, our analysis will have excluded some differential genes with low expression, some of which might be biologically interesting. For example, that spindle checkpoint dysfunction is an important cause of ANEUPLOIDY in human cancers has been suggested. The

serine-threonine kinase gene *STK6* (AURORA A)[28] might constitute a mechanism of spindle checkpoint dysregulation, and its amplification has been shown to predict resistance to taxanes.[29] Indeed, we did note differential expression between sensitive and resistant tumours—overexpression of *STK6* was about 1·4-fold higher in docetaxel-resistant tumours than in those that were sensitive to the drug (mean expression 506 and 695 in sensitive and resistant tumours, respectively; p=0·046). Nevertheless, this gene was not part of the 92-gene classifying list because of its overall low expression. This classifying list does not include all genes relevant to docetaxel sensitivity and resistance, but rather, identifies patterns of many genes that could be used as a predictive clinical test.

There is little information about the usefulness of gene expression arrays in human breast cancers.[18,30 32] Van't Veer and colleagues,[18] using printed oligonucleotide microarrays, noted that gene expression profiles were more accurate predictors of outcome in a small set of 78 young women with node-negative breast cancer than more standard clinical and histological criteria. The same investigators subsequently validated this 70-gene classifier in a cohort of 295 patients, many of whom were not in the original study.[31] The signature of poor prognosis included genes regulating cell cycle, invasion, metastasis, and angiogenesis. Perou and colleagues[32] and Sorlie and colleagues[31] used cDNA arrays and identified distinct patterns of gene expression that were termed basal or luminal. These groups differed from each other with respect to clinical outcome.[18,31] Unlike these earlier publications that dealt with patient prognosis, our aim was to identify gene expression patterns that could predict response or resistance to docetaxel in patients with primary breast cancer.

Although breast cancers are highly heterogeneous, the classifying gene list gives some clues to the mechanisms of sensitivity and resistance in some tumours. In general, resistant tumours overexpressed genes associated with protein translation, cell cycle, and RNA transcription functions, whereas sensitive tumours overexpressed genes involved in stressor apoptosis, cytoskeleton, adhesion, protein transport, signal transduction, and RNA splicing or transport. Consistent with an apoptosis-induction mode of action for taxanes, sensitive tumours had higher expression of apoptosis-related proteins (eg, BAX, UBE2M, UBCH10, CUL1). DNA damage-related gene expression in docetaxel-sensitive tumours (overexpression of CSNK2B, DDB1, ABL, and underexpression of PRKDC) also seems to contribute to docetaxel sensitivity.

Furthermore, in sensitive tumours, overexpression of genes implicated in stress-related pathways was also noted, especially heat shock proteins. Overexpression of heat shock protein 27 (HSP27) has been associated with doxorubicin resistance in the MDA-MB-231 breast cancer cell line.[33] By contrast, the same investigators have shown that HSP27-overexpressing cell lines remain sensitive to docetaxel (Fuqua S, personal communication), suggesting that different non-cross-resistant agents could have different gene patterns of sensitivity and resistance. If true, then specific patterns of gene expression could be used as tools to choose between doxorubicin and docetaxel

In a leave-one-out cross-validation procedure, the classifier that included genes selected at the nominal value of $p \leq 0{\cdot}001$ correctly classified tumours as sensitive or resistant in nearly 90% of cancers. Additionally, the predictive value of this classifier compares very favourably with that of oestrogen-receptor status, which is the only validated factor that can predict response to hormone treatment in breast cancer. Oestrogen-receptor status has a positive predictive value for response to hormone therapy of about 60%, and a negative predictive value of about 90%.[34] If about 70% of breast cancers are oestrogen-receptor positive, then sensitivity and specificity for hormone responsive and non-responsive tumours are about 93% and 50%, respectively, and the area under the ROC curve for oestrogen receptor is only about 0·72. The docetaxel classifier has positive and negative predictive values of 92% and 83%, respectively, and the area under the ROC curve of 0·96 (figure 3). Although these predictive values are likely to be slightly biased and have wide confidence intervals, these results suggest that classifiers based on gene expression would probably compare favourably with other clinically validated predictive markers.

Differences in RNA expression were confirmed by sqRT-PCR for a sample of genes. Furthermore, we have validated our classifier in an independent set of six consecutively treated patients, all of whom responded to treatment. Although the validation set is very small, it does lend support to the suggestion that gene expression arrays could be used to predict effectiveness of treatment.

This study shows that expression array technology can effectively and reproducibly classify tumours according to response or resistance to docetaxel chemotherapy. To ultimately define the molecular portrait of cancers sensitive or resistant to docetaxel, our results should be validated in a study with a large independent cohort of patients. Further patient recruitment and analysis will refine the gene list by which to classify tumours. This type of molecular profiling could have important clinical implications in defining the optimum treatment for an individual patient, thus reducing the use of unproductive treatments, unnecessary toxicity, and overall cost.

Contributors

J C Chang and E C Wooten contributed equally to this study. J C Chang designed the study, designed clinical experiments, gathered study samples, and wrote the manuscript. E C Wooten did laboratory experiments and contributed to the writing of the manuscript. A Tsimelzon and S G Hilsenbeck did statistical analysis,. M C Gutierrez, S Mohsin, and D C Allred did pathological assessment of biopsy samples. C K Osborne and R Elledge did the clincial study and wrote the manuscript. G C Chamness and P O'Connell designed the experimental studies, and wrote the manuscript.

Conflict of interest statement

J C Chang has received a Research Grant-in-Aid and is on the Speakers Bureau for Aventis. R Elledge has received a research grant from Aventis. J C Chang and P O'Connell have filed a US patent for docetaxel gene expression pattern.

Acknowledgments

We thank Mike T Lewis for his comments and manuscript review. This study was supported in part by the US Army Medical Research and Materiel Command DAMD17-01-0132, a Grant-in-Aid (US 11115) from Aventis Pharmaceutical, the Emma Jacobs Clinical Breast Cancer Fund, the Breast Cancer SPORE, P50 CA50183 from the National Cancer Institute, and the Breast Cancer Research Foundation.

References

1 The Early Breast Cancer Trialists' Collaborative Group. Systemic treatment of early breast cancer by hormonal, cytotoxic, or immune therapy: 133 randomised trials involving 31 000 recurrences and 24 000 deaths among 75 000 women. *Lancet* 1992; **339:** 1-15 (review).

2 The Early Breast Cancer Trialists' Collaborative Group. Systemic treatment of early breast cancer by hormonal, cytotoxic, or immune therapy: 133 randomised trials involving 31 000 recurrences and 24 000 deaths among 75 000 women. *Lancet* 1992; **339:** 71-85.

3 The Early Breast Cancer Trialists' Collaborative Group. Tamoxifen for early breast cancer: an overview of the randomised trials. *Lancet* 1998; **351:** 1451–67.

4 The Early Breast Cancer Trialists' Collaborative Group. Polychemotherapy for early breast cancer: an overview of the randomised trials. *Lancet* 1998; **352:** 930–42.

5 Hortobagyi GN. Docetaxel in breast cancer and a rationale for combination therapy. *Oncology* 1997; **11:** 11–15.

6 Chan S, Friedrichs K, Noel D, et al. Prospective randomized trial of docetaxel versus doxorubicin in patients with metastatic breast cancer: the 303 Study Group. *J Clin Oncol* 1999; **17:** 2341–54.

7 Nabholtz JM, Patterson A, Dirix L, et al. A phase III trial comparing docetaxel (T), doxorubicin (A) and cyclophosphamide (C) (TAC) to (FAC) as first line chemotherapy for patients with metastatic breast cancer. *Proc Am Soc Clin Oncol* 2001; **20:** 22a.

8 Henderson IC Berry D A, Demetri GD, et al. Improved disease free survival and overall survival from the addition of sequential paclitaxel but not from escalation of doxorubicin in the adjuvant chemotherapy of patients with node-positive primary breast cancer. *Proc Am Soc Clin Oncol* 1998; **17:** 101.

9 Aapro MS. Adjuvant therapy of primary breast cancer: a review of key finding s from the 8th international conference, St Gallen. *Oncologist* 2001; **6:** 376–85.

10 Fisher B, Bryant J, Wolmark N, et al. Effect of preoperative chemotherapy on the outcome of women with operable breast cancer. *J Clin Oncol* 1998; **16:** 2672–85.

11 Mamounas EP. Preoperative doxorubicin plus cyclophosphamide followed by preoperative or postoperative docetaxel. *Oncology* 1997; **11**(suppl 6)**:** 37–40.

12 Schena M, Shalon D, Davis RW, Brown PO. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* 1995; **270:** 467–70.

13 Khan J, Simon R, Bittner M, et al. Gene expression profiling of alveolar rhabdomyosarcoma with cDNA microarrays. *Cancer Research* 1998; **58:** 5009–13.

14 Lockhart DJ, Dong H, Byrne MC, et al. Expression monitoring by hybridization to high-density oligonucleotide arrays. *Nature Biotechnology* 1996; **14:** 1675–80.

15 Sgroi DC, Teng S, Robinson G, LeVangie R, Hudson JR, Elkahloun AG. In vivo gene expression profile analysis of human breast cancer progression. *Cancer Res* 1999; **59:** 5656–61.

16 Perou CM, Jeffrey SS, van de Run M, et al. Distinctive gene expression patterns in human mammary epithelial cells and breast cancers. *Proc Natl Acad Sci USA* 1999; **96:** 9212–17.

17 Whitehead Institute/MIT centre for genome research. Primer 3. http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi (accessed May 17, 2003).

18 van't Veer LJ, Dai H, van De Vijver MJ, et al. Gene expression profiling predicts clinical outcome of breast cancer. *Nature* 2002; **415:** 530–36.

19 Schadt EE, Li C, Ellis B, Wong WH. Feature extraction and normalization algorithms for high-density oligonucleotide gene expression array data. *J Cell Biochem Suppl* 2001; **37** (suppl 37)**:** 120–25.

20 Li C, Wong WH. Model-based analysis of oligonucleotide arrays: expression index computation and outlier detection. *Proc Natl Acad Sci USA* 2001; **98:** 31–36.

21 Li C, Wong WH. Model-based analysis of oligonucleotide arrays: model validation, design issues and standard error application. *Genome Biol* 2001; **2:** research0032.1-0032.11.

22 Simon R, Peng A. http://linus.nci.nih.gov/BRB-ArrayTools.html (accessed May 17, 2003).

23 Radmacher MD, McShane LM, Simon R. A paradigm for class prediction using gene expression profiles. *J Comput Biol* 2002; **9:** 505–11.

24 Ambroise C, McLachlan GJ. Selection bias in gene extraction on the basis of microarray gene-expression data. *Proc Natl Acad Sci USA* 2002; **99:** 6562–66.

25 Simon R, Radmacher MD, Dobbin K, McShane LM. Pitfalls in the use of DNA microarray data for diagnostic and prognostic classification. *J Natl Cancer Inst* 2003; **95:** 14–18.

26 NCBI. Gene expression omnibus: accession numbers GSE 349 (resistant samples) and GSE 350 (sensitive samples). www.ncbi.nlm.nih.gov/geo (accessed May 17, 2003).

27 Van Poznak C, Tan L, Panageas KS, et al. Assessment of molecular markers of clinical sensitivity to single-agent taxane therapy for metastatic breast cancer. *J Clin Oncol* 2002; **20:** 2319–26.

28 Dumontet C, Sikic BI. Mechanisms of action of and resistance to antitubulin agents: microtubule dynamics, drug transport, and cell death. *J Clin Oncol* 1999; **17:** 1061–70.

29 Anand S, Penrhyn-Lowe S, Venkitaraman AR. AURORA-A amplification overrides the mitotic spindle assembly checkpoint, inducing resistance to Taxol. *Cancer Cell* 2003; **3:** 51–62.

30 van de Vijver MJ, He YD, van't Veer LJ, et al. A gene-expression signature as a predictor of survival in breast cancer. *N Engl J Med* 2002; **347:** 1999–2009.

31 Sorlie T, Perou CM, Tibshirani R, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci USA* 2001; **98:** 10869–74.

32 Perou CM, Sorlie T, Eisen MB, et al. Molecular portraits of human breast tumours. *Nature* 2000; **406:** 747–52.

33 Hansen RK, Parra I, Lemieux P, Oesterreich S, Hilsenbeck SG, Fuqua SA. Hsp27 overexpression inhibits doxorubicin-induced apoptosis in human breast cancer cells. *Breast Cancer Res Treat* 1999; **56:** 187–96.

34 Osborne CK, Yochmowitz MG, Knight WA, 3rd, McGuire WL. The value of estrogen and progesterone receptors in the treatment of breast cancer. *Cancer* 1980; **46** (suppl 12)**:** 2884–88.